International Journal of Psychophysiology 93 (2014) 211–219

Contents lists available at ScienceDirect

International Journal of Psychophysiology

journal homepage: www.elsevier.com/locate/ijpsycho

# How stressful are 105 days of isolation? Sleep EEG patterns and tonic cortisol in healthy volunteers simulating manned flight to Mars

Angelo Gemignani [a,b,c,*], Andrea Piarulli [d,b], Danilo Menicucci [b,c], Marco Laurino [a,b], Giuseppina Rota [e], Francesca Mastorci [b,c], Vadim Gushin [f], Olga Shevchenko [f], Erika Garbella [b], Alessandro Pingitore [b,c], Laura Sebastiani [g], Massimo Bergamasco [d], Antonio L'Abbate [b,c], Paolo Allegrini [b,c], Remo Bedini [b,c]

[a] Department of Surgery, Medical, Molecular and Critical Area Pathology, University of Pisa, via Paradisa 2, 56124 Pisa, Italy
[b] EXTREME Centre, Scuola Superiore Sant'Anna, Piazza Martiri della Libertà 33, 56127 Pisa, Italy
[c] Institute of Clinical Physiology, National Research Council (CNR), via Moruzzi 1, 56124 Pisa, Italy
[d] Perceptual Robotics Laboratory, Scuola Superiore Sant'Anna, Pisa, via Alamanni 13b, 56010 Pisa, Italy
[e] Laboratory of Clinical Biochemistry and Molecular Biology, Department of Experimental Pathology, Medical Biotechnologies, Infectivology and Epidemiology, University of Pisa, via Roma 67, 56126, Pisa, Italy
[f] Institute of Biomedical Problems of Moscow, Moscow, Russia
[g] Department of Translational Research on New Technologies in Medicine and Surgery, University of Pisa, Italy

ARTICLE INFO

*Article history:*
Received 30 November 2013
Received in revised form 5 April 2014
Accepted 24 April 2014
Available online 2 May 2014

*Keywords:*
Sleep
Cortisol
Social/environmental confinement
Stress
High-density EEG
NREM sleep
MARS 500

ABSTRACT

Spaceflights "environment" negatively affects sleep and its functions. Among the different causes promoting sleep alterations, such as circadian rhythms disruption and microgravity, stress is of great interest also for earth-based sleep medicine. This study aims to evaluate the relationships between stress related to social/environmental confinement and sleep in six healthy volunteers involved in the simulation of human flight to Mars (MARS500). Volunteers were sealed in a spaceship simulator for 105 days and studied at 5 specific time-points of the simulation period. Sleep EEG, urinary cortisol (24 h preceding sleep EEG recording) and subjectively perceived stress levels were collected. Cognitive abilities and emotional state were evaluated before and after the simulation. Sleep EEG parameters in the time (latency, duration) and frequency (power and hemispheric lateralization) domains were evaluated.

Neither cognitive and emotional functions alterations nor abnormal stress levels were found. Higher cortisol levels were associated to: i) decrease of sleep duration, increase of arousals, and shortening of REM latency; ii) reduction of delta power and enhancement of sigma and beta in NREM N3; and iii) left lateralization of delta activity (NREM and REM) and right lateralization of beta activity (NREM).

Stressful conditions, even with cortisol fluctuations in the normal range, alter sleep structure and sleep EEG spectral content, mirroring pathological conditions such as primary insomnia or insomnia associated to depression. Correlations between cortisol fluctuations and sleep changes suggest a covert risk for developing allostatic load, and thus the need to develop ad-hoc countermeasures for preventing sleep alterations in long lasting manned space missions.



## 1. Introduction

Poor sleep quality has been frequently described as a common response to stress or distress (Van Reeth et al., 2000). The exposure to chronic stress often determines alterations of sleep, affecting both NREM and REM phases. NREM changes consistently observed in human studies are the increase of shallow sleep and the reduction of Slow Wave Sleep (SWS or NREM stage N3). REM changes are the increase of its duration and the shortening of the latency of the first REM period, and a general increase of REM density (Van Reeth et al., 2000). Studies both on animal models of chronic stress (Cheeta et al., 1997; Opp, 1995) and on humans (Opp, 1995) suggest that these alterations are likely mediated via corticotropin-releasing hormone (CRH) release, which in turn stimulates endogenous adreno-cortico-tropic hormone (ACTH) and cortisol secretion (i.e. hypothalamic pituitary adrenal axis – HPA – activation). HPA hyperactivation related to chronic stress is paralleled by a right hemisphere hyperarousal during wake (Hewig et al., 2008).

Astronauts involved in real or simulated long-term missions represent a model for the study of chronic stressful engagement with "real or simulated extreme environment" (Monk et al., 1998). Reduction of SWS and REM sleep, shortening of REM latency and increase of arousals are

* Corresponding author at: Department of Surgery, Medical, Molecular and Critical Area Pathology, University of Pisa, via Paradisa 2, 56124 Pisa, Italy. Tel.: +39 050 315 2686; fax: +39 050 580018.
*E-mail address:* gemignan@dfb.unipi.it (A. Gemignani).

http://dx.doi.org/10.1016/j.ijpsycho.2014.04.008
0167-8760/

typically observed in short-term space missions, due to circadian rhythm phase shifts, heightened workload demand, emergencies, and more in general, extraordinary stressful environmental conditions (Dijk et al., 2001; Mallis and DeRoshia, 2005). In this light, a crucial problem for long lasting manned missions in space is represented by sleep alterations. Sleep EEG data from long-term space missions are scarce and ground-based simulation of long lasting missions is at present the experimental condition of choice for the study of such alterations. Recently, a study investigating the relationships between sleep and long lasting spaceflight simulation has been published (Basner et al., 2013). This study, based on actigraphy and neurobehavioral assessment, identified that the simulation of long lasting space mission induces alterations of sleep quality and of sleep-wake periodicity and timing, which are mainly sustained by an inadequate circadian entrainment.

Here we report a study in which the model of stress is represented by the simulation of the human flight to Mars (*MARS105*, pilot phase of the *MARS500* project). Six healthy volunteers were kept for 105 days within an isolation facility mimicking a spacecraft and were studied during the flight simulation as well as right before and after the period of confinement. In this condition, stress was represented by social and environmental confinement, enforced interaction between crewmembers, resources rationing, limited and lagged communications with the outside and environmental acoustic noise.

The working hypothesis of the study addresses the issue of stress vulnerability to spatial confinement and social isolation in a super-selected and well-trained population. This pilot study is the first international attempt aimed at identifying indices of abnormal stress responses in healthy volunteers in order to design and develop ad hoc countermeasures to be administered in the study of 520 days of isolation (MARS 500), which represents the real simulation of human flight to Mars. The aim of the study was to examine how stress, measured by tonic cortisol levels and subjectively perceived stress levels, affects sleep in terms of structure and EEG power/lateralization and whether it produces long lasting effects on cognitive abilities and emotional state that persist beyond the confinement duration.

As in many simulation studies with such complex experimental setups (Basner et al., 2013; Schneider et al., 2010), the choice of an appropriate statistical analysis has been conditioned by two important issues concerning the high number of parameters necessary for sleep characterization and the involvement of a small number of participants and sampling times. In order to override these intrinsic limitations, a hierarchical approach aimed at pruning the numerous variables that characterize sleep patterns was employed in the data analysis. Principal Component Analysis (PCA) and confirmatory ANCOVAs performed on the selected variables allowed the identification of significant associations between specific sleep features and cortisol levels, considered by consensus a reliable indicator of stress.

## 2. Materials and methods

### 2.1. Subjects selection and experimental setup

Six healthy right-handed male volunteers (mean age 33 ± 6 years) have been selected for a 105-days (from March 31st to July 14th, 2009) mission to Mars simulation organized by the Institute for Biomedical Problems (IBMP, Moscow, Russia) and the European Space Agency (ESA). Crewmembers selection procedures, based on a thorough psychophysical evaluation of the candidates, were the same of those used for real astronauts (for further details on the selection procedure see Supplementary material). The protocol of the study was approved by the Ethics Committee of the IBMP and by the ESA Medical Board, and was compliant with all guidelines stated in the Declaration of Helsinki. All participants gave informed consent to participate in the study.

Crewmembers were sealed in an isolation facility that simulated a spacecraft flight for a 105-day period (the real round trip to Mars will last approximately 500 days). The isolation facility was artificially lighted (16 h on, 8 h off), ambient temperature was maintained constant at 24 °C, and an environmental noise in line with that of the International Space Station (60–70 dB) was constantly present (see Supplementary material for further details). Once sealed into the facility, the crewmembers had only personal contact with each other and a time-limited voice contact with a simulated control center was the only connection with the outside. Inside the isolation facility, daily tasks were organized in order to match those typical of human spaceflight missions. The crew had a working schedule similar to the one of space crews: 8 h of work, 8 h of free time and 8 h of sleep. They had to perform scientific experiments dealing with the psychological and physiological effects of confinement. They also had to monitor life support systems and keep the standards of chambers hygiene. Mars Mission impact was introduced via resources rationing, characterized by no re-supply from outside and communication limitations with the outside world (communication was conducted with a realistic time delay of up to 25 min, to simulate the communications lag between Mars and Earth). The crewmembers were under 24-hours of outside supervision, and the group of supervisors on duty consisted of a medical doctor, a medical assistant, an engineer and his assistant.

The design of our experiment included 5 time-points:

- BDC: Basal Data Collection, performed pre-simulation (27th to 29th of March).
- T1, T2, T3: three equally spaced time points during the 105 days simulation (T1, 22nd to 24th of April, T2, 27th to 29th of May, and T3, 30th of June to 2nd of July).
- RDC: Recovery Data Collection performed post-simulation (19th to 21st of July).

Sleep and stress levels were evaluated at each time-point, while the neuropsychological evaluation was performed only during BDC and RDC.

As no person was allowed to enter the simulation module from the outside for the 105 days of confinement, crewmembers had to carry the experiments by themselves as normally happens during space flights. To this aim, the crew underwent a training phase before the beginning of the simulation, in order to learn the correct procedures of the experiment setup and as a support, each subject was provided with a logbook containing a step-by-step description of all the experimental procedures. In order to keep the experimental procedure unchanged at all time-points, sleep EEG recordings were conducted by crewmembers also during BDC and RDC phases.

### 2.2. Cognitive and emotional neuro-psychological evaluation (BDC, RDC)

A clinical psychologist evaluated working memory, attention and mood state immediately before (BDC) and immediately after (RDC) the 105 days of isolation. The choice of this cognitive and emotional evaluation was based on previous literature showing an effect of confinement on these specific functions (Hockey and Sauer, 1996; Dèchamps and Rosnet, 2005). Cognitive functions were evaluated by means of three subsets of the Wechsler Memory Scale (Wechsler, 1997): Digit Span for numeric working-memory function, Picture Arrangement for logic temporal reasoning and Digit Symbol Coding for attention. Visuospatial working memory was evaluated with the Corsi block test (Vandierendonck et al., 2004), whereas spatial reasoning was tested through the Kohs Cubes test (Kohs, 1923). Finally, the Profile of Mood States (POMS) (McNair et al., 1992) was administered to evaluate six factors: tension–anxiety, depression–dejection, anger–hostility, fatigue–inertia, vigor-activity and confusion–bewilderment. The whole evaluation lasted approximately one hour and a half and took place in the medical division of the isolation facility.

### 2.3. Stress levels evaluation (BDC, T1, T2, T3, RDC)

Urinary cortisol samples were collected at each time-point (BDC, T1, T2, T3, RDC) in the 24 h immediately preceding the sleep EEG recording (from 8 pm of the day before to 8 pm of the day of the sleep recording). Since urine produced in the 24 h was collected in a single flask, only the all-day cortisol concentration was available. Tonic cortisol levels were measured using urinary free cortisol test-kit DKO018, Lot 1730 from DIAMETRA, Milan, Italy. The Perceived Stress Scale (PSS) questionnaire (Cohen et al., 1983) was auto-administered during daytime at each time point.

### 2.4. Sleep EEG recordings and processing (BDC, T1, T2, T3, RDC)

At each time point, volunteers went to sleep at their scheduled bedtime and whole night high-density (128 channels, Electrical Geodesics, Eugene, OR) sleep EEG recording was performed. The use of a high-density EEG (128 channels) allowed overcoming the low skill of volunteers in the EEG montage. Indeed, the lack of any experimental control on this phase of EEG recording (the montage) induced us to choose a high-density EEG system that allowed a redundancy of electrodes with the aim of obtaining a significant number of electrodes with a good signal quality. The high-density system comprised also electrodes for eye movement and zygomatic muscle tone recordings.

Signals referenced to Cz were acquired with a sampling rate of 250 Hz and with electrode impedances kept below 50 kΩ. Offline signal processing and statistical analyses were implemented using Matlab (MathWorks, Natick, MA, USA). EEG raw data were notch-filtered to remove power-line frequency and re-referenced to the average potential of the mastoids.

Movement artifacts were detected according to Menicucci et al., 2009, and Piarulli et al., 2010. After a confirmatory visual inspection, most of them (95%) were discarded. Sleep stages were visually scored in line with American Academy of Sleep Medicine criteria (Iber et al., 2007). EEG analysis was limited to the first two sleep cycles, due to the decline of signal quality (progressive drying of the EEG electrodes from the third cycle on), which was apparent in most of the recordings. Each sleep EEG recording was characterized with time-domain and frequency domain parameters. For the time domain we estimated the following features: from the first sleep cycle, the N3 and REM Latency; averaging over the cycles 1 and 2, the time duration of sleep phases (N2 time, N3 time, REM time and Wakefulness after Sleep Onset time — WASO time), the total sleep time (the sum of N2, N3 and REM times) and the frequency of stage shifts and arousals. Stage Shift was defined as a change of sleep stage in line with Borbély et al., 1985; arousal was defined as an abrupt shift of EEG frequency including alpha and/or higher frequencies (with the exception of spindles) that lasts at least 3 s, with at least 10 s of stable sleep preceding the change (Borbély et al., 1985).

For the frequency-domain parameters, in each sleep stage we estimated single channels EEG power spectra related to six EEG frequency bands (Fast Fourier Transform calculated and averaged on consecutive 20 s epochs): delta (0.5–4 Hz, Slow Wave Activity, SWA), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–15 Hz), beta (15–25 Hz) and gamma (25–90 Hz). For each band, we estimated two parameters: (i) Band Median Power (BMP) obtained as the median power value over scalp electrodes; (ii) Band Hemispheric Lateralization (BHL), defined as the difference between right and left hemispheres median power spectra, excluding thus mid-line electrodes from the computation (48 electrodes for each hemisphere were retained). Power band measures were expressed in dB.

### 2.5. Statistical procedures

In order to overcome the issue of low sample size-to-variable ratio we applied a hierarchical approach that pruned the sleep-related parameters and then we calculated the association between the remaining ones and the two stress measures (cortisol and PSS levels). This association was tested after correction of the factors (subject and time of sampling) putatively influencing the measures (step illustrated in the upper panel of Fig. 1). Repeated measures ANOVA and one-way ANOVA were used in order to check for, respectively, time and subject effects on each parameter. The correction was applied only in case of significant factor effect. As no variable showed any significant time effect, no correction was applied. In contrast, limited to frequency-domain parameters, the subject effect was significant and thus removed.

After these corrections, the analyses of possible relations between stress and sleep parameters were conducted considering each experimental point statistically independent from the others.

Besides the study of the stress versus sleep feature associations, we also compared the neuropsychological scores collected before and after the flight simulation by using the non-parametric paired Wilcoxon Signed Rank Test.

#### 2.5.1. Pruning and identification of stress-sleep associations

Sleep variables were divided into 7 datasets: one in the time-domain, six in the frequency domain, that is BMP and BHL estimated in N2, N3 and REM stages for each frequency band. The pruning procedure was applied separately on each dataset. Moreover, the two stress measures were added to each dataset in order to retain only sleep variables sharing information with them.

The variable pruning procedure was based on the PCA. Many rules of thumb about either the minimum sample size $S$ or the sample size-to-variable ratio ($S$:$v$) have been proposed for PCA to produce stable solutions (Kline, 1979; Cattell, 1978). In order to obtain reliable results even from sample sizes less than 50 a general consent about a $S{:}v \geq 5$ has been reached (Barrett and Kline, 1981; Gorsuch, 1983). As all the datasets had $S{:}v$ below the minimum of 5 (range 2.5 to 3.75), we had to reduce to six the number of variables submitted to each PCA.

The criterion used for dropping sleep variables from each dataset was the Measure of Sampling Adequacy (MSA, Kaiser, 1970) of each variable; MSA indicates whether the variable shares an adequate level of variance with the other ones of the same dataset, including the stress measures (Hair et al., 2010).

The PCA pruning procedure (Fig. 1, middle panel) works as follows:

- PCA is performed on the entire dataset.
- MSA is calculated for each variable.
- Variables with MSA < 0.5 are excluded from the dataset (Kaiser, 1970).
- If the number of retained variables still exceeds six, the first two steps are repeated.

If the pruning yielded low MSA for both PSS and cortisol, the analysis was stopped since no reliable correlation between stress parameters and other parameters was found; if, on the contrary, at least one among cortisol and PSS was retained, the final PCA was performed.

The results of the final PCA were considered reliable if the following three conditions (Hair et al., 2010) were met (see Fig. 1, middle panel):

a) The pruned dataset satisfied Bartlett's test of sphericity.
b) The MSA of each retained variable was higher than 0.5.
c) The overall Kaiser–Meyer–Olkin measure of sampling adequacy was higher than 0.5 (Kaiser, 1970).

For each PCA satisfying the three conditions, the number of retained principal components was estimated applying Horn's Parallel Analysis (Hayton et al., 2004). For each retained component, the loadings were extracted. Component loadings are the correlation coefficients between each variable and the component. When two or more variables have significant loadings on the same component, this provides indication of the existence of a common underlying process contributing to the variables behavior.

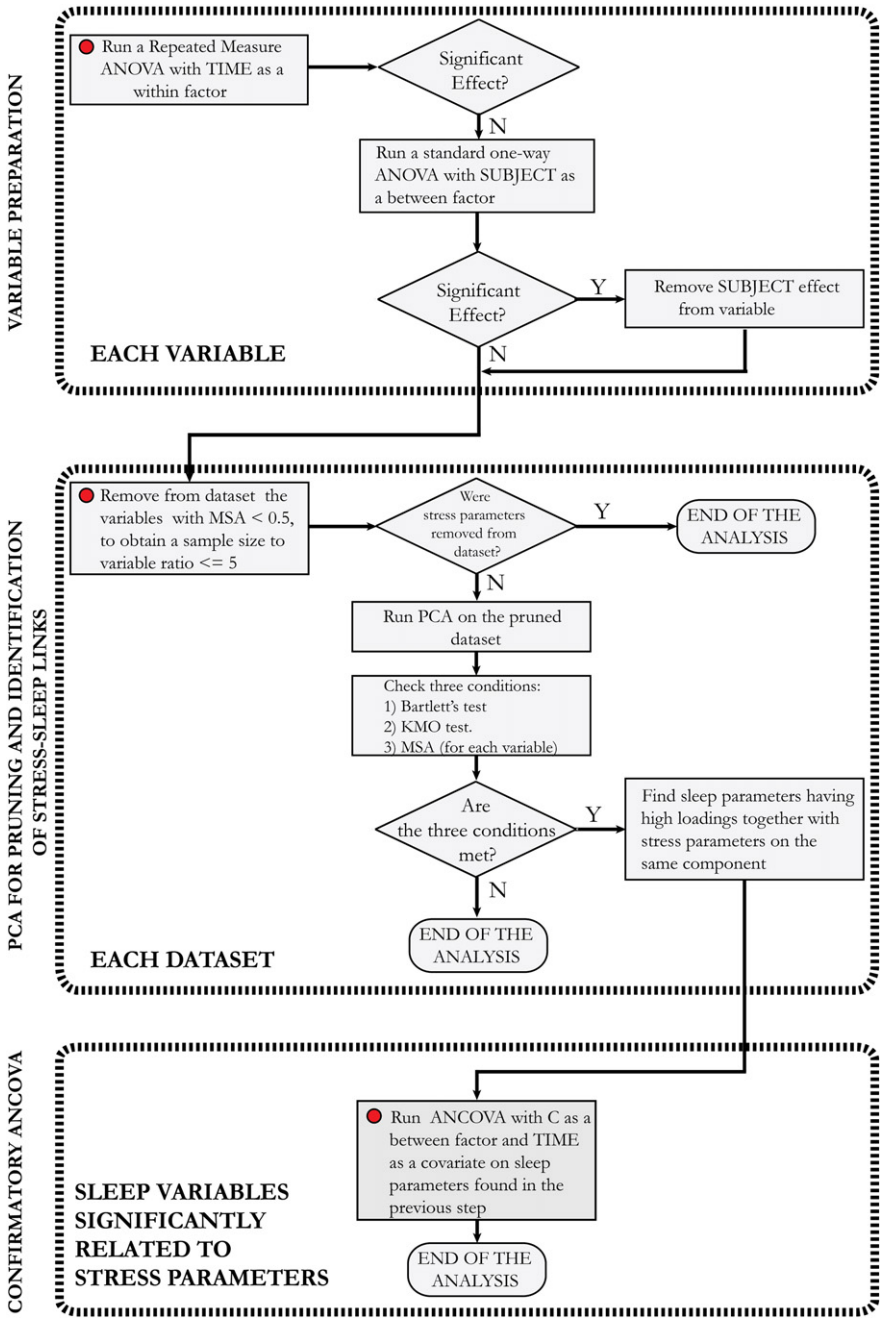


**Fig. 1.** Flowchart of data analysis steps. Upper panel describes the variable preparation (checking of Time and subject effects on each variable). Note that the repeated measures ANOVA with time effect checkbox has no "Y" output as no variable showed significant effects. Middle panel describes both the pruning (described in more detail in the Materials and methods section, Pruning and identification of stress-sleep associations procedure, subsection) and the final PCA procedures applied to each dataset. Lower panel describes the confirmatory ANCOVA with C as a between factor and time as covariate applied to variables selected by PCA. A Red dot indicates, for each of the three panels, the starting block of the flowchart.

*2.5.2. Confirmatory analysis of stress-sleep associations*

All variables stood Shapiro–Wilk test for normality (Shapiro and Wilk, 1965), thus confirmatory statistical analyses were performed using parametric statistics (step illustrated in the lower panel of Fig. 1). As a confirmatory step, planned ANCOVAs with cortisol treated as a dichotomous variable C and time (treated as an ordinal variable) as covariate were conducted on those sleep parameters that on the basis of PCA were significantly related both to cortisol and PSS. In order to obtain two equally populated groups (low C and high C), threshold for cortisol dichotomization was set at the median value of the distribution. Cortisol and not PSS was chosen for confirmatory analyses of variance, as the former is a more objective measure of stress level and of HPA axis activation. Also, a substantial agreement of objective (cortisol) and subjectively perceived (PSS) stress levels was demonstrated both by the high positive correlation between them ($r = 0.59$, $p < 0.001$) and by ANCOVA performed on PSS with C as a between factor and time as covariate ($F_{1,27} = 8.4$, $p < 0.01$).

Relations between cortisol and sleep parameters were considered significant only if they were verified both in PCA and ANCOVA models. The rationale of this approach relies on the use of exploratory PCA as a hypothesis generator (i.e. PCA findings were tested by planned ANCOVA with C as a between factor). No correction for multiple comparison was applied on ANCOVAs as each test was conducted to confirm or reject findings derived from PCA.

## 3. Results

### 3.1. Cognitive and emotional neuro-psychological evaluation

The comparison of psychometric scores did not reveal significant differences between BDC and RDC phases either for cognitive abilities or for emotional state (see Table 1).

### 3.2. Stress and sleep time-domain parameters

Table 2 summarizes descriptive statistics of stress and sleep time-domain parameters related to each subject. Note that crewmembers showed on average: (i) perceived stress scale (PSS) scores lower than normative levels (Cohen and Williamson, 1988); (ii) cortisol levels within the normality range (Görges et al., 1999), and (iii) a higher amount of REM sleep in the first two sleep cycles associated to a shortening of REM latency of the first REM period.

The PCA pruning procedure yielded six retained variables: PSS, cortisol, sleep time, arousal frequency, N3 time and REM latency.

PCA applied to stress and pruned sleep time-domain parameters resulted in one retained PCA component. Component loadings are depicted in Fig. 2, panel A. Cortisol and PSS were associated since they had significant loadings on the same principal component, thus we could consider them as a unitary marker of stress (the same holds true for all the other frequency domain datasets).

As shown in Fig. 2, higher stress (cortisol and PSS) levels yield: higher arousal frequency, reduction of sleep time, and shortening of REM latency. Confirmatory one-way ANCOVAs with 2-levels C and time as covariate were performed on the retained variables after pruning, and results are presented in Fig. 3. Coherently with PCA findings, significant negative relations with C were found for REM latency ($F(1,27) = 4.2$, $p < 0.05$) and sleep time ($F(1,27) = 5.4$, $p < 0.05$) while a positive relation with arousal frequency ($F(1,27) = 4.3$, $p < 0.05$). The time effect was not significant, thus confirming the results of the repeated measures ANOVA performed in order to correct the variables for the time factor putatively influencing the measures. Time effect was not significant also for the frequency domain sleep features.

### 3.3. Stress and N2 spectral parameters

Regarding BMP datasets, PSS and cortisol had MSA below 0.5, and as such were not suitable for deriving reliable factors associating stress to N2 B.P. Accordingly, no confirmatory ANCOVAs were conducted on BMP dataset (Fig. 4).

Regarding BHL dataset, the pruning procedure yielded four retained frequency bands: delta, theta, alpha, and beta. The PCA yielded one component showing significant direct correlations with cortisol and beta, indicating that the higher was the cortisol level the greater was the right lateralization for beta (increasing BHL). The same component showed inverse correlations with delta, theta and alpha (Fig. 2C, left diagram) indicating that the higher was the cortisol level, the greater was the left lateralization for delta, theta and alpha (decreasing BHL). Even though the comparison of BHLs between high and low C levels showed lateralization changes (Fig. 5) in line with the PCA results, the confirmatory ANCOVAs did not yield any significant C effect.

### 3.4. Stress and N3 spectral parameters

Retained variables for BMP were cortisol, PSS, delta, sigma, beta and gamma, whereas for BHL were cortisol, PSS, delta, theta, alpha and beta. PCAs yielded one component both for BMP and BHL datasets.

The BMP component was directly correlated with cortisol, PSS and frequencies from sigma to beta and gamma and inversely with delta (Fig. 2B).

The BHL component was directly related to cortisol, PSS, beta, and inversely to delta, theta and alpha (Fig. 2C, central diagram). Higher cortisol and PSS levels were associated to a shift towards right BHL for beta, while delta, theta and alpha to a shift towards left BHL.

Confirmatory ANCOVAs with C as between-factor were conducted on BMP and BHL retained variables (variables framed in red in Fig. 4 for BMP and in Fig. 5 for BHL).

As for BMP, high C had lower values for delta ($F(1,27) = 4.4$, $p < 0.05$), and higher values for sigma ($F(1,27) = 7.9$, $p < 0.01$) and beta ($F(1,27) = 4.6$, $p < 0.05$) than low C.

As for BHL, high C exhibited a higher left lateralization for delta ($F(1,27) = 8.2$, $p < 0.01$) and theta ($F(1,27) = 6.6$, $p < 0.05$) and a higher right lateralization for beta ($F(1,27) = 6.3$, $p < 0.05$) than low C.

### 3.5. Stress and REM spectral parameters

Regarding the BMP dataset related to REM, no analyses were conducted as MSA of PSS and cortisol were both below 0.5.

With regard to REM BHL the pruning procedure selected delta, sigma, beta and gamma bands. PCA performed on the pruned dataset had one significant component that yielded direct correlations with cortisol, PSS, sigma, beta and gamma on one side, and inverse correlation with delta (Fig. 2C, right diagram). Similarly to what observed for N3 data, higher cortisol levels were related to a right lateralization for high frequency bands, and a left lateralization for the delta band. Confirmatory ANCOVA, applied on delta, sigma, beta and gamma BHL, indicated a significant C-effect for delta, showing a shift towards left at High C, ($F(1,27) = 6.4$, $p < 0.05$) (Fig. 5).

## 4. Discussion

We investigated the relationships between stress, sleep, cognitive abilities and emotional state in six male volunteers enrolled in the MARS500 pilot phase and sealed within an isolation facility module for 105 days.

**Table 1**
Cognitive and emotional test scores descriptive statistics for BDC, column 4, and RDC, column 5. Values are presented as mean ± standard error. Last column represents results of Wilcoxon paired test on each of the parameters.

| Functions | Test | Subtest | BDC | RDC | Statistics |
|---|---|---|---|---|---|
| Numeric working memory | Digit span | Forward | 6.6 ± 1.36 | 7.0 ± 0.63 | NS |
| | | Backward | 5.5 ± 1.76 | 6.6 ± 0.81 | NS |
| Spatial working memory | Corsi's blocks | – | 6.8 ± 0.98 | 6.5 ± 0.83 | NS |
| Logic temporal reasoning | Picture arrangement | – | 13.0 ± 2.44 | 11.16 ± 2.48 | NS |
| Attention | Digit symbol coding | – | 17.0 ± 1.67 | 17.8 ± 1.47 | NS |
| Spatial reasoning | Kohs cubes | – | 7.74 ± 0.10 | 7.79 ± 0.07 | NS |
| Mood quality | Profile of Mood States — POMS | Tension–anxiety | 47.0 ± 8.83 | 36 ± 2.94 | NS |
| | | Confusion–bewilderment | 42.0 ± 5.29 | 35.1 ± 2.92 | NS |
| | | Depression–dejection | 46.16 ± 5.81 | 43.0 ± 2.19 | NS |
| | | Fatigue–inertia | 46.33 ± 6.25 | 40.0 ± 4.64 | NS |
| | | Anger–hostility | 45.0 ± 8.94 | 40.6 ± 1.2 | NS |
| | | Vigor-activity | 57.6 ± 5.39 | 61.1 ± 9.26 | NS |

**Table 2**
Descriptive statistics of stress and sleep time-domain parameters for each crew member evaluated across the 5 time-points (mean ± standard error, values are expressed in minutes unless otherwise stated). All sleep parameters are related to the first two sleep cycles except for N3 latency and REM latency, which refer to the first cycle.

| Features/subjects | Subject 1 | Subject 2 | Subject 3 | Subject 4 | Subject 5 | Subject 6 |
|---|---|---|---|---|---|---|
| Cortisol (mg/24 h) | 126 ± 54 | 126 ± 65 | 159 ± 73 | 141 ± 48 | 56 ± 45 | 92 ± 32 |
| PSS (scores) | 8.2 ± 3.1 | 7.6 ± 1.8 | 9.6 ± 3.8 | 8.0 ± 2.1 | 6.4 ± 3.0 | 4.8 ± 0.8 |
| Sleep time | 140 ± 43 | 162 ± 26 | 166 ± 39 | 157 ± 15 | 209 ± 27 | 167 ± 35 |
| WASO time | 11 ± 8 | 7 ± 6 | 34 ± 25 | 13 ± 6 | 40 ± 39 | 43 ± 42 |
| Arousal frequency (n/min) | 0.14 ± 0.11 | 0.04 ± 0.03 | 0.02 ± 0.01 | 0.10 ± 0.04 | 0.02 ± 0.01 | 0.04 ± 0.02 |
| Stage shifts frequency (n/min) | 0.49 ± 0.27 | 0.19 ± 0.06 | 0.17 ± 0.05 | 0.37 ± 0.10 | 0.22 ± 0.07 | 0.16 ± 0.05 |
| N2 time | 57 ± 27 | 73 ± 34 | 68 ± 17 | 60 ± 20 | 123 ± 29 | 78 ± 9 |
| N3 time | 48 ± 20 | 54 ± 20 | 65 ± 15 | 56 ± 20 | 55 ± 11 | 56 ± 18 |
| REM time | 25 ± 5 | 35 ± 11 | 34 ± 18 | 40 ± 14 | 29 ± 5 | 33 ± 21 |
| N3 latency | 14 ± 1 | 16 ± 1 | 12 ± 3 | 12 ± 2 | 15 ± 2 | 16 ± 2 |
| REM latency | 60 ± 18 | 49 ± 21 | 83 ± 34 | 69 ± 14 | 108 ± 35 | 71 ± 14 |

The principal result of this study is the identification of relationships between stress markers and specific sleep macrostructure features as well as EEG rhythms.

Crewmembers showed perceived stress scale (PSS) scores lower than normative levels (Cohen and Williamson, 1988) and cortisol levels within the normality range (Görges et al., 1999); these two stress markers were highly correlated. PSS accounts for a subjective perception of stress related to the last month, while tonic cortisol level refers to the 24 h before sleep recording, thus the robust correlation between these parameters suggests a long range eustress effect on daily cortisol level.

We found that higher cortisol levels are paralleled by a shortening of REM latency and a reduction of sleep time of the first two sleep cycles in the following night, together with an increased occurrence of arousals. A lowering of delta activity and an increase of sigma and of wake-like EEG beta accompanied these changes during N3 sleep. The same associations were found between PSS and the pattern of band powers during N3, showing that, as previously observed in patients affected by primary insomnia (Hall et al., 2000), subjectively perceived stress is linked to alterations in the EEG power distribution during N3. Moreover higher cortisol levels of the 24 h before EEG recordings were associated to: (i) a right lateralization of beta and a left lateralization of delta bands during NREM sleep, and (ii) a left lateralization of delta band during REM sleep.

Relationships between human sleep structure and HPA axis under physiological and pathophysiological conditions, as well as effects on sleep of systemically administered HPA hormones have been extensively investigated in the past years (Friess et al., 1995), and mutual relationships between the temporal patterns of HPA axis hormones and sleep stages were already described in the eighties (Born et al., 1986; Steiger et al., 1987). It has been observed that Slow Wave Activity reaches its climax during the first part of the night when cortisol is low and growth hormone is at its maximum, whereas the second half of the night is characterized by a preponderance of REM sleep, low growth hormone concentration and progressively higher levels of cortisol. Born et al. (1986) underlined that epochs of wakefulness and light sleep were associated with increased plasma cortisol concentrations. Actually, a decrease of Slow Wave Sleep (SWS) duration, and a subjective perception of poorer sleep have been associated to stressors related to working conditions (Kecklund and Akerstedt, 2004). Among stress hormones, corticotropin-releasing hormone (CRH) could play an important role in the observed interaction between cortisol and sleep patterns (Ehlers et al., 1986; Holsboer et al., 1988; Opp et al., 1989; Opp, 1995; Steiger, 2007; Tsuchiyama et al., 1995).

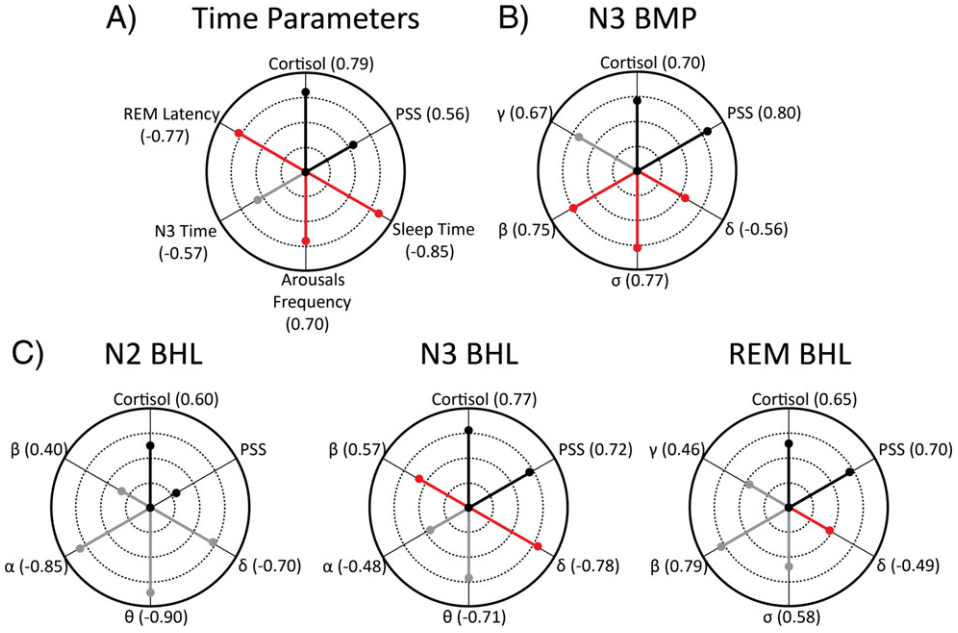


**Fig. 2.** PCA loading diagrams for time-domain parameters (panel A), N3 BMP (panel B), N2, N3 and REM BHL (panel C). For BHL loading diagrams, a direct correlation between a BHL and cortisol indicates a shift towards right hemispheric prevalence at higher cortisol levels. For each PCA only the component significantly related both to cortisol and sleep parameters is depicted. In each diagram, radii represent loading values of the parameters; significant loadings are represented by thick lines and the corresponding correlations values are enclosed in brackets. In each loading diagram, variables submitted to the ANCOVA with C as a between-factor are highlighted in red if the ANCOVA yielded a significant C effect, in gray if no significant effect was found.

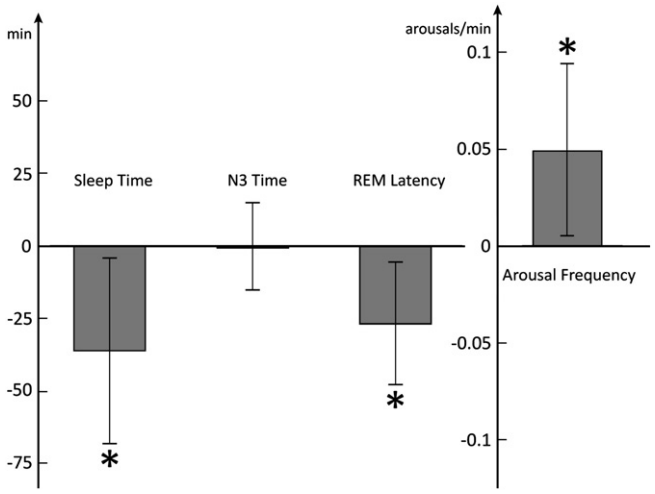


**Fig. 3.** Descriptive statistics (mean and 95% confidence interval) of high–low C differences are presented for time parameters selected from PCA. Each selected parameter was submitted to an ANCOVA with C as a between factor and time as a covariate (asterisks denote significance at $p < 0.05$).

Findings regarding sigma activity deserve, in our opinion, a separate discussion: the enhancement of sigma at higher cortisol levels seems at odds with a primary role of spindles in memory consolidation (Destexhe et al., 2007), which is classically counterbalanced by glucocorticoids (Wagner and Born, 2008). However, Marshall et al. (2006) demonstrated that association between boosting of declarative memory and enhancement of spindles activity is limited to the low frequency interval (8–12 Hz) that corresponds to our alpha band. Other authors pointed out a sleep protective function of spindles especially towards auditory stimuli (Dang-Vu et al., 2011). In our experimental environment, which was highly contaminated by acoustic noise, a higher spindle activity at higher cortisol levels could have had a sleep-preserving role. At higher cortisol levels the brain is less asleep, as apparent from lower delta activity and higher wake-like activity expression, and a partial inhibition of thalamic sensory gating occurs (Steriade and McCarley, 2010); in this sense spindle activity could be elicited to suppress auditory stimuli conveyed to the cortex. Another interpretation of our findings can be drawn from the following: our analysis, focusing on a spectral representation of the EEG signals, does not discriminate between spindle oscillations and sigma activity, so that our sigma could be part of a broadband wake-like activity including beta (Menicucci et al., 2013). In our opinion, evidence of links between HPA axis and sleep sigma expression described in previous works could be interpreted in this framework. Andrew et al. (2002), found a lowering of delta and an increasing of sigma and beta in patients with subjective insomnia when compared to normal subjects. Moreover, Antonijevic et al. (1999) showed how CRH administration in healthy subjects enhanced EEG power in the sigma and low-beta range both during wakefulness and SWS.

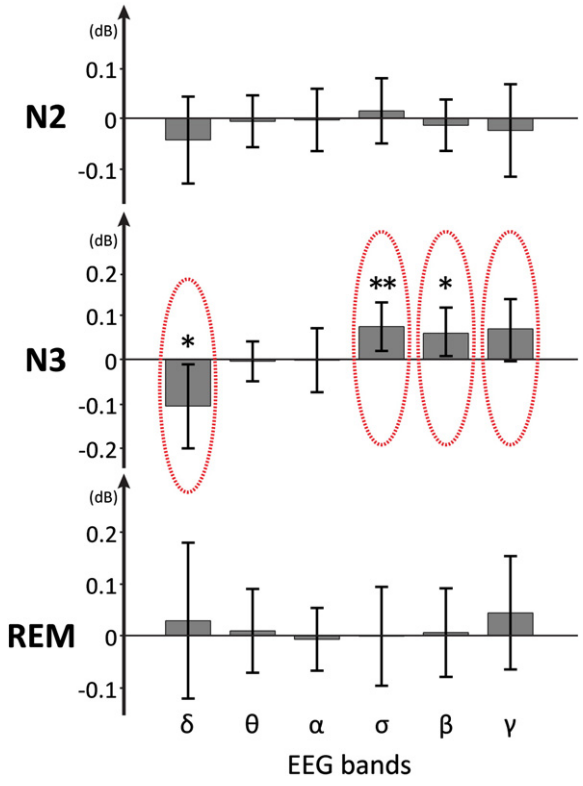


**Fig. 4.** Descriptive statistics (mean and 95% confidence interval) of high–low C differences are presented for BMP variables. Upper, middle and lower panels refer to N2, N3 and REM sleep BMP respectively. BMP variables on which the confirmatory ANCOVA with C as a between factor and time as a covariate was conducted (selected on the basis of PCA) are encircled by dotted lines. Asterisks denote significant ANCOVAs (single asterisk, $p < 0.05$; double asterisks, $p < 0.01$).

Several lines of evidence suggest, during wakefulness, links between right hemisphere lateralization and human stress response both in healthy subjects (Hewig et al., 2008) and in depressive patients (Hecht, 2010). Our findings of a right hemispheric prevalence during NREM sleep for beta activity at higher cortisol seem consistent with this scenario and the aroused right hemisphere can be regarded as an adverse environment for sleep. On the other hand, delta power level showed a left hemispheric prevalence at higher cortisol during NREM sleep, consistent with the offline reprocessing related to the previous highly demanding wakefulness (Poe et al., 2010). This could indicate that mild cortisol levels produce a eustress condition able to support the emotional arousal via activation of the right hemisphere in the beta band, while maintaining the cognitive functions via the Slow Wave Activity expression in the left hemisphere. Accordingly, several animal models show that mild cortisol increases stimulate, rather than depress, the hippocampal neurogenesis, which is involved in learning consolidation processes (Lucassen et al., 2010). Also, evidence in humans shows that high cortisol could improve memory consolidation of salient information over a night of sleep (Bennion et al, 2013).

In contrast with a whole body of literature (for a review see Vandekerckhove and Cluydts, 2010), no link was found between any band median power during REM sleep and cortisol. However, due to experimental issues, analyses were limited to the first two sleep cycles, while the greater expression of REM sleep appears later in the night. We cannot exclude effects on REM sleep when REM sleep is at its zenith (Payne and Nadel, 2004).

We found a delta left hemispheric prevalence during REM sleep in the same direction of that found during NREM sleep; however, it is worth noting that delta activity during REM sleep has to be considered as a part of the theta rhythm instead of being sustained by a specific thalamo-cortical oscillator as during NREM sleep (Steriade and McCarley, 2010).

Finally, no effect of prolonged confinement was found either for cognitive abilities or for emotional state suggesting that the light sleep alterations associated to high, yet physiological, levels of cortisol are not sufficient to impair brain functions. This finding is in agreement with Schneider et al. (2010), demonstrating in the framework of the same simulation that mood of crewmembers, after a decrease during the first 11 weeks, returned to baseline levels during the last week of isolation. The lack of any alteration of emotional status seems at odds with the shortening of REM latency identified in our subjects at higher cortisol levels. Notice that similar shortenings of REM latency and durations of REM sleep in the first two sleep cycles (Table 2) are typically observed in depression (Steiger and Kimura, 2010). Indeed, these changes have been considered either a marker of cholinergic over-activity in

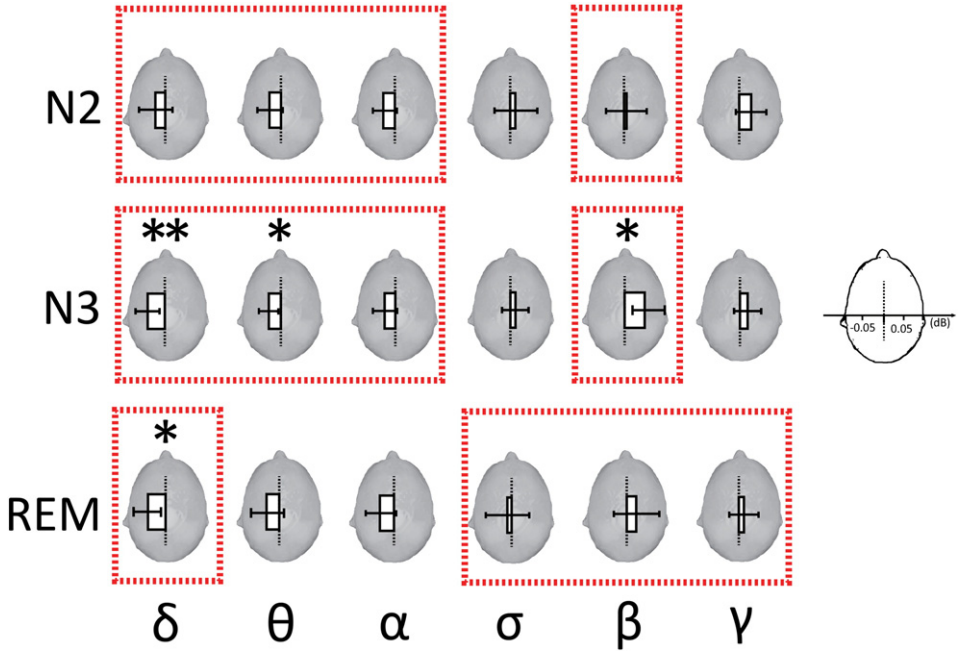


**Fig. 5.** Descriptive statistics (mean and 95% confidence interval) of high–low C differences are presented for BHL variables. Upper, middle and lower rows refer to N2, N3 and REM sleep BHL respectively. Each head plot represents results related to a specific band. In each head plot, a bar plot of the high C–low C mean difference for the corresponding BHL is presented. Error bars denote 95% confidence intervals for the mean. BHL variables on which the confirmatory ANCOVA with C as a between factor and time as a covariate was conducted (selected on the basis of PCA) are enclosed in dotted rectangles. Asterisks denote significant ANCOVAs (single asterisk $p < 0.05$; double asterisk $p < 0.01$). The scale used for each head-plot is depicted in the head-plot at the end of middle panel.

depression (Palagini et al., 2013) or a marker of stressful reaction, as shown in animals exposed to chronic mild stress (Cheeta et al., 1997).

The described findings highlight a physiological relationship between total cortisol production related to the previous 24-hour activity and sleep of the following night. Even if related only to the first two sleep cycles, these findings overlap those found both in animal and human models of chronic stress and can be interpreted in the framework of well-known interactions between HPA axis and sleep (Steiger, 2007). The lack of any information about the quality of sleep in the nights preceding the cortisol measurement, does not allow to completely exclude sleep restriction effects on HPA axis and thus on cortisol levels (Leproult et al., 1997). In other words, we are not able to discriminate whether cortisol alters sleep or vice versa or, even more, if our results reflect the establishment of a self-sustaining vicious cycle. According with our working hypothesis, i.e. identifying a stress vulnerability, the direction of the association between cortisol and sleep is not pivotal since it is the association per se to play a key role for designing possible ad hoc non pharmacological countermeasures (e.g. transcranial direct current stimulation) able to prevent sleep alterations and maintain sleep memory functions (Marshall et al., 2006). In line with several reports of NASA, ESA and Roscosmos, sleep dysfunctions are able to jeopardize the real spatial mission, increasing the risk of incidents and accidents (Mallis and DeRoshia, 2005). Despite the study of the association between cortisol and sleep is not novel when cortisol is abnormal, it becomes original when cortisol fluctuates in the normal range. This appears crucial for super-selected and well-trained subjects engaged in extreme long-lasting spatial missions.

As a caveat, we stress that our results might not apply to general population because of the small sample size and the peculiarity of our sample. Indeed, this is a study for preparing humans to fly to Mars. Nevertheless, our results are in line with a more common response of sleep to stressful conditions associated with an increase in cortisol levels, typically observed in animal models and in general population.

In conclusion, we believe that 105 days of simulation do not represent a stressor able to produce an allostatic load with associated cognitive or emotional alterations but rather a eustress condition useful to produce an adaptive response characterized by increased arousal to external stimuli and maintained sleep homeostatic functions.

We cannot exclude that longer simulations, such as 500 days corresponding to the real duration of a flight to Mars, could elicit an allostatic load (McEwen, 2012). In this sense, we believe that our findings could contribute to define indicators of astronauts "well-being" that may be monitored during space missions and to design possible ad hoc countermeasures.

## Acknowledgments

This work is dedicated to the memory of Prof. Mario Guazzelli.

This research was supported by Italian Space Agency (ASI) through grant ASI no. I/006/09/0

We thank the Institute of Biomedical Problems (IBMP, Moscow, Russia) and the European Space Agency (ESA) for the organization of the 105 day simulation.

We thank Luca Bastiani and Marco Scalese of Social Epidemiological Section of the Institute of Clinical Physiology of the National Research Council for revising the statistical procedures.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijpsycho.2014.04.008.

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